

## CLAIMS

1. A method detecting biomolecules, where

(a) the biomolecules to be detected are coupled to a first substance which is part of a nucleic acid replication device,

5 (b) the formed biomolecules/substance complexes are bound to the solid phase bound binding molecules that are specific for the particular biomolecules,

(c) if called for the non-bound biomolecules/substance complexes are removed by washing,

10 (d) the bound biomolecules/substance complexes are incubated with high molecular weight nucleic acid molecules and mononucleotides of various species of which at least the nucleotides of one species are fitted with a detectable marking, further with a second substance which complements the first substance into a functional replicating device for high molecular weight nucleic acids, said device binding the high molecular weight nuclear acid molecules and under integration of marked mononucleotides generating replicas of the high molecular weight nucleic acid molecules and mononucleotides that do not dissociate off it,

15 (e) if called for, removing by washing the dissolved high molecular weight nucleic acid molecules and mononucleotides,

(f) and determining the biomolecules to be detected from the detection of the marked replicas.

20

2. A method detecting biomolecules, where

(a) immobilized biomolecules are incubated with connecting complexes consisting of binding molecules specific to the particular biomolecules and of a first substance that is part of a nucleic acid replication device,

25 (b) if called for removing by washing the non-bound connection complexes,

(c) incubating the formed biomolecule/complexes with high molecular weight nucleic acid molecules and mononucleotides of various species, at least the mononucleotides of one species being fitted with a detectable marking and with a second substance that comple-

ments the first substance coupled to the biomolecules into a functional, replicating device for high molecular weight nucleic acids, said device binding the high molecular weight nucleic acid molecules and generating under integration of marked mononucleotides replicas of the high molecular weight nucleic acid molecules which do not dissociate off it,

5 (d) if called for removing by washing the dissolved nucleic acid molecules and mononucleotides,

(e) and determining the biomolecules to be detected by detecting the marked replicas.

10 3. Method for detecting biomolecules as claimed in claim 2, characterized in that

(a) prior to incubation with the connection complexes, the biomolecules are immobilized by being bound to solid phase bound specific binding molecules,

(b) following incubation of the biomolecules with the connection complexes, the non-bound connection complexes are removed by washing with water if called for,

15 (c) prior to detecting the marked replicas, the dissolved high molecular weight nucleic acid molecules and mononucleotides are removed by washing if called for.

4. Method for detecting biomolecules as claimed in claim 1, characterized in that the biomolecules to be detected are covalently coupled to the first substance.

20 5. Method for detecting biomolecules as claimed in either of claims 2 and 3, characterized in that, in the connection complexes, the binding molecules are covalently coupled to the first substance.

25 6. Method for detecting biomolecules as claimed in claim 1, characterized in that the biomolecules to be detected are coupled by linker systems to the first substance.

7. Method for detecting biomolecules as claimed in either of claims 2 and 3, characterized in that, in the connection complexes, the binding molecules are coupled by means of linker system to the first substance.

5 8. Method for detecting biomolecules as claimed in either of claims 6 and 7, characterized in that the linker system is selected from the group containing the biotin/streptavidin system, the ULS platinum linker system, the digoxigenin system, an antigen/antibody system or another specifically binding system.

10 9. Method for detecting biomolecules as claimed in one of claims 1 through 8, characterized in that the first substance contains the  $\beta$  sub-unit of a DNA polymerase III and the second substance contains the remaining, required sub-units of a DNA polymerase III.

15 10. Method for detecting biomolecules as claimed in one of claims 1 through 8, characterized the first substance is one or several sub-units of a DNA polymerase III and the second substance contains  $\beta$  sub-units of a DNA polymerase III and, where called for, further required sub-units of a DNA polymerase III.

20 11. Method for detecting biomolecules as claimed in one of claims 1 through 8, characterized in that the first substance contains  $\beta$  sub-units of a DNA polymerase III and the second substance is a DNA polymerase I, the Klenow fragment of a DNA polymerase I, the Taq DNA polymerase or another DNA polymerase.

25 12. Method for detecting biomolecules as claimed in one of claims 1 through 8, characterized in that the first substance is a DNA polymerase I, the Klenow fragment of a DNA polymerase I, the Taq DNA polymerase or another DNA polymerase and the second substance contains  $\beta$  sub-units of DNA polymerase III.

13. Method for detecting biomolecules as claimed in one of the above claims, characterized in that, in addition to the second substance, the biomolecule connection complex(es) (is) are also incubated with further  $\beta$  sub-units of a DNA polymerase III.

5 14. Method for detecting biomolecules as claimed in one of the above claims, characterized in that the high molecular weight nucleic acid molecules to be replicated are circular in shape.

10 15. Method for detecting biomolecules as claimed in one of the above claims, characterized in that the high molecular weight nucleic acid molecules each comprise one replication origin sequence.

15 16. Method for detecting biomolecules as claimed in one of the above claims, characterized in that high molecular weight nucleic acid molecules are at least 10 kb long.

17. Method for detecting biomolecules as claimed in one of the above claims, characterized in that the detectable marking of at least one of the added mononucleotide species consists of a fluorescent, a luminescent, a radioactive or an enzymatic marking.

20 18. Method for detecting biomolecules as claimed in one of the above claims, characterized in that the solid phase bound binding molecules are mounted on a biochip.

19. Method for detecting biomolecules as claimed in claim 18, characterized in that the marked replicas are detected using biochip scanners.

25 20. Method for detecting biomolecules as claimed in one of claims 1 through 17, characterized in that the solid phase bound binding molecules are mounted on beads.

21. Method for detecting biomolecules as claimed in claim 20, characterized in that the marked replicas are detected using flow detectors.

22. Method for detecting biomolecules as claimed in one of claims 1 through 17, characterized in that the solid phase bound binding molecules or the immobilized biomolecules are configured in a biological preparation.

23. Method for detecting biomolecules as claimed in claim 22, characterized in that the biological preparation is a histological section, a freeze rupture preparation or a Western Blot.

24. Method for detecting biomolecules as claimed in one of the above claims, characterized in that the biomolecules to be detected are amino acids, proteins, sugars, nucleic acids, antibodies, lectins, lipids or receptors.

25. Method for detecting biomolecules as claimed in one of the above claims, characterized in that the binding molecules are proteins, sugars, nucleic acids, antibodies, lectins, receptors or other specifically binding molecules.

26. A method for preparing a marker for the purpose of detecting biomolecules, where

a first substance which is part of a nucleic acid replicating device and which comprises a coupling element,

is incubated with high molecular weight nucleic acid molecules and mononucleotides of different species, of which at least the mononucleotides of one species are fitted with a detectable marking, also with a second substance that complements the first substance into a functional replicating device for high molecular weight nucleic acids, in such manner that

the device so formed binds the high molecular weight nucleic acid molecules, and, while integrating marked mononucleotides, does generate replicas of the high molecular weight nucleic acid molecules that do not dissociate off it.

5           27.     Method for preparing a marker for the purpose of detecting biomolecules as claimed in claim 26, characterized in that the coupling element is a functional group able to covalently bind with molecules to be bound.

10           28.     Method for preparing a marker for the purpose of detecting biomolecules as claimed in claim 26, characterized in that the coupling element is part of a linker system allowing to bind the molecules to be bound.

15           29.     Method for preparing a marker for the purpose of detecting biomolecules as claimed in claim 28, characterized in that the linker system is selected from the group comprising the biotin/streptavidin system, the ULS/platinum linker system, the digoxigenin system, an antigen/antibody system or another specifically binding system.

20           30.     Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 29, characterized in that the first substance is connected by the coupling element to a binding molecule able to specifically binding a biomolecule.

25           31.     Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 29, characterized in that the first substance is connected by the coupling element to a biomolecule.

32.     Method for preparing a marker for the purpose of detecting biomolecules as claimed in claim 30, characterized in that the binding molecules are proteins, sugars, nucleic acids, antibodies, lectins, receptors or other specifically binding molecules.

33. Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 30 through 32, characterized in that the biomolecules to be detected are amino acids, proteins, sugars, nucleic acids, antibodies, lectins, lipids or receptors.

5

34. Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 33, characterized in that the first substance is the  $\beta$  sub-unit of a DNA polymerase and the second substance contains the remaining, required sub-units of a DNA polymerase III.

10

35. Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 34, characterized in that the first substance is one or more sub-units of a DNA polymerase III and the second substance contains  $\beta$  sub-units of a DNA polymerase and any further required sub-units of a DNA polymerase III.

15

36. Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 34, characterized in that the first substance contains  $\beta$  sub-units of a DNA polymerase III and the second substance is a DNA polymerase I, the Klenow fragment of a DNA polymerase I, the Taq DNA polymerase or another DNA polymerase.

20

37. Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 34, characterized in that the first substance is a DNA polymerase I, the Klenow fragment of a DNA polymerase I, the Taq DNA polymerase or another DNA polymerase and the second substance contains  $\beta$  sub-units of a DNA polymerase III.

25

38. Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 34, characterized in that, in addition to the particular second substance used, the first substance is incubated with further  $\beta$  sub-units of a DNA polymerase III.

5

39. Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 38, characterized in that the high molecular weight nucleic acids to be replicated are circular in shape.

10

40. Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 39, characterized in that the high molecular weight nucleic acid molecules to be replicated each comprise one replication origin sequence.

15

41. Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 40, characterized in that the high molecular weight nucleic acid molecules are at least 10 kb long.

20

42. Method for preparing a marker for the purpose of detecting biomolecules as claimed in one of claims 26 through 41, characterized in that the detectable marking of at least one of the added mononucleotide species consists of a fluorescent, luminescent, radioactive or enzymatic marking.

43. A marker detecting biomolecules, characterized in that it is prepared by a method defined in claims 26 through 42.